

**Patent Claims**

1. Method for fluorescence microscopy, especially with a laser scanning microscope in which an at least partially spectrally resolved detection of the fluorescence spectrum occurs and reference spectra are used for spectrally demixing, characterized by the fact that recording of the reference spectra occurs from temporally and/or spectrally variable dyes and/or dye combinations and serves for image evaluation.
2. Method according to Claim 1 for recording of organic processes.
3. Method according to Claim 2 for recording of intracellular processes.
4. Method according to Claim 2 for recording of intercellular processes.
5. Method according to one of Claims 1 to 4 for recording of cells and/or cell populations.

It is to be understood that the present invention is not limited to the illustrated embodiments described herein.

Modifications and variations of the above-described embodiments of the present invention are possible, as appreciated by those skilled in the art in light of the above teachings. It is therefore to be understood that, within the scope of the appended claims and their equivalents, the invention may be practiced otherwise than as specifically described.

What is claimed is:

1. A method using fluorescence microscopy for image

evaluation, the method comprising the steps of:

using a laser scanning microscope in which an at least  
partially spectrally resolved detection of the fluorescence  
spectrum occurs;

using reference spectra for spectral demixing;

employing temporally and/or spectrally variable dyes and/or  
dye combinations for recording of the reference spectra; and  
demixing the recorded reference spectra for image  
evaluation.

2. The method according to claim 1 wherein the method records  
organic processes.

3. The method according to claim 1 wherein the method records  
intracellular processes.

4. The method according to claim 2 wherein the method records  
intercellular processes.

5. The method according to claim 1 wherein the method records  
cells and/or cell populations.

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5. ~~Method~~The method according to one of ~~Claims~~claim 1 to 5 in which recording of reference spectra of photoconvertible dyes occurs.

7. ~~Method~~The method according to one of ~~Claims~~claim 1 to 7 in which recording of reference spectra of photoactivatable dyes occurs.

8. ~~Method~~The method according to one of ~~Claims~~claims 1 to 7 in which recording of reference spectra of indicator dyes occurs.

9. ~~Method~~The method according to one of ~~Claims~~claim 1 to 8 in which recording of reference spectra of dyes occurs that change their spectra dynamically based on intracellular processes.

10. ~~Method~~The method according to one of ~~Claims~~claim 1 to 9 in which recording of reference spectra of dyes occurs with a different rise in fluorescence intensity.

11. ~~Method~~The method according to one of ~~Claims~~claims 1 to 10 in which recording of reference spectra of the fluorescing protein Kaede occurs.

12. ~~Method~~The method according to one of ~~Claims~~claims 1 to 11 in which recording of reference spectra of PA-GFP occurs.